REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	4/7/97	Final Report 1/15/93 - 12/31/95

4. TITLE AND SUBTITLE

Inhibition of olfactory receptor cells

5. FUNDING NUMBERS

N00014-90-J-1566

6. AUTHOR(S)

Barry W. Ache and Robert M. Greenberg

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Whitney Laboratory
University of Florida
9505 Ocean Shore Blvd.
St. Augustine FL 32086

8. PERFORMING ORGANIZATION REPORT NUMBER

N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

Office of Naval Research
800 N. Quincy St.
Arlington VA 22217

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

N/A

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Distribution unlimited

13. ABSTRACT (Maximum 200 words)

CDNAs encoding two elements of the inositol phosphate-based intracellular signaling system(an IP_3R, as well as a related $G\alpha_q$ protein) were fully cloned and sequenced from the olfactory organ of the spiny lobster, an aquatic animal model for olfactory research. Antibody and molecular probes developed from these sequences are being used to study how this one of two olfactory signaling pathways is distributed across the ensemble of receptor cells that constitute the olfactory organ. The resulting insight into peripheral olfactory organization could improve the design of detector arrays in biosensors for odors and other chemical substances.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Olfaction, Transduction, Ion Channel, Biosensor	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	UL

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	**PR**	- Project
G	- Grant	**TA**	- Task
PE	- Program Element	**WU**	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

- **DOD** - See DoDD 5230.24, "Distribution Statements on Technical Documents."
- **DOE** - See authorities.
- **NASA** - See Handbook NHB 2200.2.
- **NTIS** - Leave blank.

Block 12b. Distribution Code.

- **DOD** - Leave blank.
- **DOE** - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
- **NASA** - Leave blank.
- **NTIS** - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

FINAL PROGRESS REPORT

Grant #: N00014-90-J-1566 R&T Code: 341r002

PRINCIPAL INVESTIGATORS: Barry W. Ache and Robert M. Greenberg

INSTITUTION: University of Florida

GRANT TITLE: Inhibition of olfactory receptor cells: an aquatic model

AWARD PERIOD: January 15, 1993 - December 31, 1995

OBJECTIVE: To develop molecular probes for two second-messenger activated ion channels involved in olfactory transduction, and to use these probes to study how the two signaling pathways are distributed across the ensemble of receptor cells that constitute the olfactory organ.

APPROACH: Molecular biology.

ACCOMPLISHMENTS: In the prior period of support, we showed that cultured lobster olfactory receptor neurons (ORNs) express several types of second messenger-activated ion channels - two activated by IP_3, one activated by IP_4 and a fourth activated by cAMP. In the present period of support, we initiated a molecular biological effort to begin to develop molecular probes with which to study the distribution of one or more of these ion channels across the receptor cell population. Initial success in cloning a fragment of a cAMP-activated channel from lobster nose RNA could not be sustained in order to obtain the full sequence due to doubts about the authenticity of the fragments. Instead, we pursued the suggested structural similarities between lobster plasma membrane $InsP_3$-gated channels (IP_3Rs) and known intracellular IP_3Rs in other systems to clone an IP_3R. We amplified a partial cDNA, homologous to known IP_3Rs, from reverse transcribed lobster olfactory organ RNA using degenerate primers and the polymerase chain reaction (PCR). We extended the clone to the 3'-noncoding region using 3'-RACE. A variety of techniques were used to isolate the remaining substantial 5'end, including construction and screening of IP_3R mini-cDNA libraries and 5'-RACE. We have now fully cloned and sequenced cDNAs encoding an IP_3R, as well as a related $G\alpha_q$ protein, from the spiny lobster olfactory organ. The IP_3R cDNA has an 8409 bp open reading frame coding for 2803 amino acids (a computed 320 kDa protein), and is homologous to known IP_3Rs (50-61% amino acid identity). The very rare message has been localized to both the olfactory organ and the brain using an RNAse protection assay. The $G\alpha_q$ cDNA has an open reading frame of 1059 bp (353 aa); the sequence is homologous to known $G\alpha q$ proteins (70-83% amino acid identity). The computed

molecular weight of the protein is 41.5 kDa, consistent with that of the native protein. Northern analysis demonstrates a 4.5 kDa $G\alpha_q$ message in olfactory organ. We are in the process of localizing these molecules to the transduction zone of the cells using antibodies raised to specific sequences.

CONCLUSIONS: Our results provide the basis for developing molecular probes to explore the distribution of two components of the inositol phosphate signaling pathway in lobster olfactory receptor cells.

SIGNIFICANCE: Having multiple effectors in ORNs opens up the interesting and novel possibility that not all ORNs express the same effector(s) and that differences in effector expression may define the existence of functionally different classes of ORNs within the receptor cell population. Such an organizational strategy could have consequence for the design of the detector array in biosensors for odors or other chemical signals.

PATENT INFORMATION: N/A

AWARD INFORMATION: N/A

PUBLICATIONS AND ABSTRACTS:

Publications:

Munger, S.D, Rust, N.R, Wiese, E and Ache, B.W. Molecular characterization of an olfactory organ Gq protein. (In preparation).

Munger,S.D., Wiese, E., Ache, B.W. and Greenberg, R.M. Molecular and immunocytochemical characterization of an olfactory organ IP_3 receptor. (In preparation).

Abstracts:

Munger, S.D., B.W. Ache and Greenberg, R.M. (1995). Cloning and sequencing of an IP_3-receptor from lobster olfactory organ. Unpublished Abstract, Gordon Research Conference on Calcium Signals.

Munger, S.D., Ache, B.W. and Greenberg, R.M. (1995). Cloning and sequencing of an IP_3-receptor cDNA from lobster olfactory organ. Soc. Neurosci. Abstr. 21:1127.

Munger, S.D., B.W. Ache and Greenberg, R.M. 1(1995). Cloning and sequencing of an IP_3-receptor partial cDNA from lobster olfactory organ. Chem. Senses. 20:748.

Munger, S.D., Rust, N.C., Ache, B.W. and Greenberg, R.M. (1996). Cloning and molecular characterization of two components of the IP_3 pathway from lobster olfactory organ. Chem. Senses 21:648.